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# Unilateral Injection of Neuropeptide Y Decreases Blood Flow in the Injected Testis but May Also Increase Blood Flow in the Contralateral Testis

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**ABSTRACT:** Neuropeptide Y (NPY) receptors have recently been described in intratesticular arterioles, but the role of NPY in testicular blood-flow regulation has not been examined. To explore this, we administered NPY in various doses (0.01–10 μg) via intratesticular injections and studied testicular microcirculation using a laser Doppler flow meter. NPY injection shows a dose-response pattern, with 1 μg (the most potent dose) causing a decrease (−42.4 ± 3.7%, $P < 0.00005$) in blood flow in the ipsilateral testis of all the animals and an increase in blood flow in the contralateral testis (+17.2 ± 5.6%, $P = 0.03$, $n = 25$ animals). The response in the contralateral testis was variable. A clear-cut increase was seen in 19 of the 25 animals examined, whereas either no response or a slight decrease was seen in the remaining six. The contralateral increase, which was not seen in the hindpaw on the same side, did not occur when the neuronal connections to the testes were blocked by injection of local anesthetics into the spermatic cord, either on the NPY-injected side or on the contralateral side. Our results suggest that NPY may serve as a vasoconstrictor in the testis, probably by acting on the NPY-Y1 receptors present on intratesticular arterioles. Local injection of NPY causes a major decrease in blood flow in the injected testis. This decrease is followed in the majority of animals studied by an increase in blood flow in the contralateral testis, an effect that seems to depend on neuronal mechanisms. This observation suggests that the testes may communicate under certain situations. The functional consequences of this remain to be elucidated.

Key words: NPY, innervation.

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Neuropeptide Y (NPY), a 36–amino acid polypeptide (Tamamoto et al, 1982), is one of the most abundant and ubiquitous neuropeptides in the mammalian nervous-system. It is expressed in neurons in both the central and the peripheral nervous system, and it is often colocalized with noradrenaline (Lundberg et al, 1983). NPY is involved in the control of neuroendocrine and behavioral functions (Clark et al, 1984, 1985; Kalra et al, 1990, 1992). The synthesis and release of NPY in the hypothalamus is regulated by testicular steroids (Sahu et al, 1992). High concentrations of NPY are observed in the male genital tract (Adrian et al, 1984), and NPY-containing nerve fibers are widely distributed throughout the male genitalia (Fahrenkrug et al, 1989). NPY-containing nerves reach the testis via the superior (along the testicular artery) and inferior spermatic nerves (Allen et al, 1989; Properzi et al, 1992; Rauchenwald et al, 1995; Zhu et al, 1995), and NPY-containing fibers are seen around subcapsular blood vessels but not inside the testis (Setchell et al, 1994). Leydig and Sertoli cells may also express NPY mRNA, and the expression is increased by luteinizing hormone (LH) and interleukin (IL)-1α (Kanzakiet al, 1996). In addition, recent studies have demonstrated NPY-Y1 receptors in the muscular layer of intratesticular arteries (Kopp et al, 1997), and, in other tissues, NPY causes vasoconstriction by acting on this receptor (Kim et al, 1994). Therefore, the aim of this study was to further investigate the role of NPY in the regulation of testicular blood flow (TBF).

## Materials and Methods

### Materials

NPY (7180) was purchased from Peninsula Laboratories (Belmont, California). Lidocaine hydrochloride (Xylocain®, Astra, Sweden) was used as local anesthetic.

### Animals

Adult male Sprague-Dawley rats (300–400 g) purchased from Möllegaard (Denmark) were held in standard laboratory conditions with food and water *ad libitum*. Anesthesia of the rats (pentobarbital, 50–60 mg/kg, intraperitoneal injection) preceded the experiments. To maintain a stable body temperature (37°C) during procedures, the animals were kept supine on a heating pad. The design of this study was approved by the local animal ethics committee in Umeå, Sweden.

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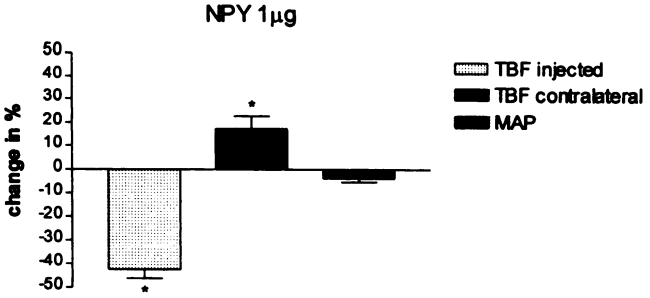


**FIG 1.** Effects of intratesticular injection of neuropeptide Y (NPY), 1 μg, on testicular blood flow (TBF) and mean arterial pressure (MAP). Injections were made into the left testis. Error bars indicate standard error of the mean. * Significantly different ($P < 0.05$) from corresponding values in NaCl-treated rats ($n = 25$).

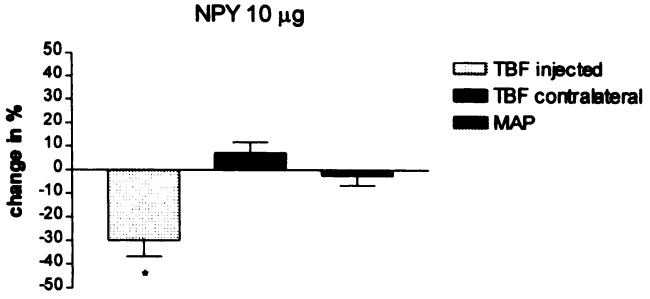


**FIG. 2.** Effects of intratesticular injection of neuropeptide Y (NPY), 10 μg, on testicular blood flow (TBF). Injections were made into the left testis. Error bars indicate standard error of the mean. * Significantly different ($P < 0.05$) from corresponding value in NaCl-treated rats ($n = 8$).

### *Blood Flow and Mean Arterial Pressure (MAP)*

The scrotal sacs were opened, and, to immobilize the testes, they were placed in small, open plastic cups containing melted agar, as described elsewhere (Collin et al, 1996b). TBF was measured during periods of 5 minutes prior to and 15 minutes after the injections in both testes simultaneously, by use of a two-channel laser Doppler flowmeter, PF 4001 master (Perimed AB,Stockholm, Sweden) and two multireceiver probes, PF 412 (PerimedAB). The probes were held with micromanipulators approximately 1mm over the testicular surfaces and 5 mm below the cranial pole in an area devoid of large vessels. MAP was measured through a canula inserted in the carotid artery by use of a pressure transducer (SensoNor 840, ULAB, Bromma, Sweden) connected to a nisolated pressure amplifier (Lectromed 5290/5291, ULAB). MAP was registered throughout each experiment. The laser Doppler flow signals and the MAP were recorded and later analyzed by use of a personal computer (Compac Prolinea 4/25) and the software Perisoft v. 5.0 (Perimed AB).

### *Experiment 1*

Various doses of NPY (0.01 μg [$n = 5$], 0.1 μg [$n = 5$], 1 μg [$n = 25$], and 10 μg [$n = 8$]) were used. NPY was dissolved in 50 μl sterile saline (0.15 M NaCl) and injected into the caudal pole of the left testis by use of a 27G hypodermic needle. Blood flow was measured approximately 10 mm proximal to the site of injection. Five additional animals were injected with saline, as controls.

### *Experiment 2*

To block a possible neuronal regulatory system affecting the contralateral testis, 0.1 ml of xylocain (20 mg/ml) was administered around the spermatic cord, 10 mm above the cranial pole, by use of a 27G needle. The anesthesia was applied 10 minutes prior to the injection of NPY (1.0 μg), either on the same side ($n = 7$) as the NPY injection or on the contralateral side ($n = 5$).

### *Experiment 3*

NPY (1 μg) was administered in one testis as described above ($n = 7$), and blood flow was monitored simultaneously in the injected testis and in the contralateral hindpaw.

### *Statistics*

Statistical assessment was performed by means of the Mann-Whitney *U*-test. Values are given as mean ± standard error of the mean (SEM). A $P$ value <0.05 was considered statistically significant.

## *Results*

### *Experiment 1*

Injection of saline caused an increase in TBF of 6.8 ± 5.8% in the injected testis and 0.5 ± 2.8% in the contralateral testis. MAP in the saline-treated animals showed a decrease of -1 ± 1.8%.

When compared with saline, injection of 0.01 ($n = 5$) and 0.1 μg ($n = 5$) NPY did not significantly influence TBF in the testicular tissue located approximately 10 mm from the injection site. MAP and TBF in the contralateral testes were also unaffected.

Injection of 1 μg NPY ($n = 25$) caused a marked and prolonged decrease (−42.4 ± 3.7%) in TBF and had an inhibiting effect on vasomotion in all the injected testes (Fig. 1). In the contralateral testis, TBF (+17.2 ± 5.6%, $P = 0.03$) was found to be significantly increased when the whole group was analyzed (Fig. 1). However, the contralateral response was variable. A clear-cut increase (+24.6%) was observed in 19 of the animals, but, in the remaining 6 animals, blood flow was either unaffected or slightly decreased (−14.4%). In both testes, the effects of 1-μg NPY injections appeared immediately and lasted for at least 15 minutes. The response in the contralateral testis was not correlated with the magnitude of the response in the injected testis or with MAP. MAP (−3.7 ± 1.6%) was not significantly influenced by local injection of 1.0 μg NPY.

Injection of 10 μg ($n = 8$) NPY caused a marked and prolonged decrease in TBF (−29.8 ± 6.8%) in the injected testis (Fig. 2). In the contralateral testis, TBF (+7.1 ± 4.5%) was not significantly changed for the group analyzed as a whole (Fig. 2). However, as for the 1.0-μg

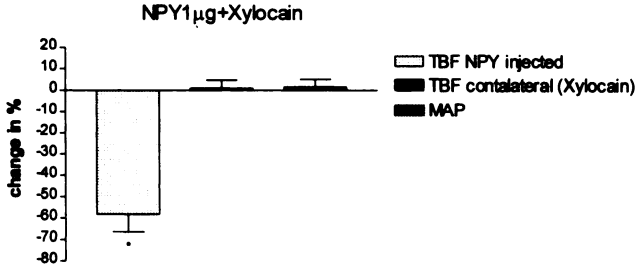


**FIG. 3.** Effects of injection of neuropeptide Y (NPY) into the left testis on left and right testicular blood flow (TBF) when local anesthesia was applied around the right spermatic cord. Error bars indicate standard error of the mean. * Significantly different ($P < 0.05$) from corresponding value in NaCl-treated rats ($n = 5$).

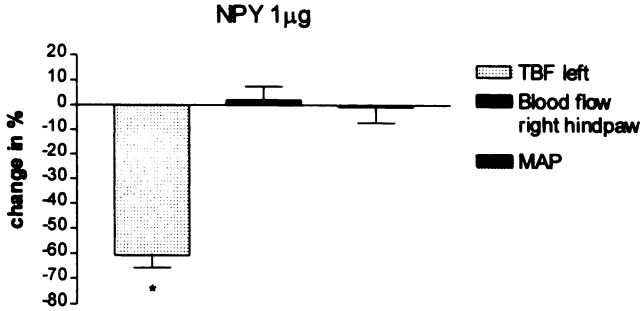


**FIG. 4.** Effects of injection of neuropeptide Y (NPY), 1 μg, on testicular blood flow (TBF), blood flow in the right hindpaw, and mean arterial pressure (MAP). Injection was made in the left testis. Error bars indicate standard error of the mean. * Significantly different ($P < 0.05$) from corresponding values in NaCl-treated rats ($n = 7$).

dose, the response in the contralateral testis in the individual rats was variable. An increase (+14.4 ± 4.3%) was observed in five of the animals, a slight decrease (−7.5 ± 1.5%) was observed in two animals, and one animal remained unaffected. MAP increased (+7 ± 2.3%) in four of the animals and decreased (−11.8 ± 4.6%) in the remaining four. The changes in MAP were not correlated with the changes in TBF.

### *Experiment 2*

When Xylocain was administered to the right spermatic cord and NPY injected into the left testis ($n = 5$), TBF in the NPY-injected testis showed a marked decrease (−58.2 ± 8.1%), TBF in the contralateral testis was unaffected (+1.0 ± 3.7%), and MAP did not change significantly (+1.6 ± 3.4%) (Fig. 3). Administering Xylocain to the left spermatic cord and injecting NPY into the left testis ($n = 7$) resulted in a distinct decrease (−55.6 ± 7.0%) in TBF in the ipsilateral testis, no significant change (−3.3 ± 10.9%) in TBF in the contralateral testis, and a decrease (−16.7 ± 8.1%) in MAP. That is, when Xylocain was administered to either the right or the left spermatic cord, prior to the injection of NPY, the increase in TBF in the contralateral testis, demonstrated in experiment 1, was blocked.

### *Experiment 3*

Injection of 1 μg NPY into the left testis caused a distinct decrease in TBF in the ipsilateral testis (−60 ± 4.9%), similar to the results in experiment 1. Simultaneous measurement of blood flow in the right hindpaw showed that this was unaffected (+2 ± 5.5%, $n = 7$; Fig. 4). This experiment was performed to rule out the possibility that systemic factors caused the results in experiment 1.

## Discussion

This study demonstrates that NPY is a potent vasoconstrictor in the testis. The effect could be mediated via the NPY-Y1 receptors present in the muscular layer of intratesticular arterioles (Kopp et al, 1997). Sympathetic NPY-containing nerve fibers have been observed in the subcapsular interstitium and around blood vessels (Properzi et al, 1992; Rauchenwald et al, 1995), and, as neuropeptides may diffuse over wide areas and exert long-lasting actions (Lundberg, 1996), it is possible that NPY released from subcapsular nerves may influence testicular blood vessels. In line with this, it has been demonstrated that sympathetic denervation increases and sympathetic stimulation decreases TBF (Setchell et al, 1994). NPY could therefore be a major component in the sympathetic neuronal control of TBF. Other possible intratesticular sources of NPY are the Leydig and Sertoli cells (Kanzaki et al, 1996), and both of these cell types are situated close to intratesticular blood vessels (Bergh and Damber, 1993). It is also possible that circulating NPY derived from either adrenal medullary chromaffin cells (Lundberg et al, 1983) or from platelets (Ericsson et al, 1987, 1991) could influence testicular blood vessels. Another possibility is that NPY, instead of having a direct effect, potentiates norepinephrine-induced vasoconstriction, as has been shown in other vascular beds (Lundberg et al, 1996). To elucidate the mediation of NPY actions in the testis, further studies have to be made.

While studying the local effects of NPY in the testis, we made an unexpected observation: injection of 1.0 μg NPY caused a clear-cut increase in blood flow in the contralateral testis in 19 of the 25 animals studied. This effect is probably selective to the testis, since no effect whatsoever was observed in the contralateral hindpaw. The mechanism behind this increase is unknown. One possibility is that it could be induced by an increase in systemic blood pressure, since testis blood flow is closely related to blood pressure (Lissbrant et al, 1997), but this does not seem to be the case, as systemic blood pressure was unaffected. The effect occurred rapidly and could therefore be neuronally mediated. This hypothesis is supported by the observation that blocking either afferent nerves from

the injected testis or efferent nerves to the contralateral testis inhibited the contralateral response. Of interest, recent studies suggest that the testis receive significant contralateral and bilateral innervation (Rauchenwald et al, 1995). This innervation pattern thus forms the necessary preconditions for a putative cross-talk between the testes, and a major decrease in blood flow in one testis may induce a neuronal reflex, causing bilateral testicular vasodilatation. The effect of such a reflex might be too weak to restore flow in the NPY-injected testis where vasoconstriction predominates, but strong enough to increase flow in the contralateral organ, at least in the individual animals with a prominent bilateral innervation pattern. This hypothesis requires that the testis receive vasodilatory nerves. This is actually the case, as vasoactive intestinal peptide (VIP)– and calcitonin gene-related peptide (CGRP)–containing nerves are observed in the testicular capsule (Setchell et al, 1994; Zhu et al, 1995) and both CGRP (Lissbrant et al, 1997) and VIP (Lissbrant and Bergh, 1997) are able to increase TBF.

The observation that blood-flow changes in one testis may influence the other is also in line with the observation, reported elsewhere, that unilateral testicular torsion influences blood flow in the contralateral testis (Tanyel et al, 1989; Kizilcan et al, 1992; Akgur et al, 1993). In that situation, however, blood flow was decreased in the contralateral testis. However, in the unilateral varicocele model, there is a bilateral increase in TBF (Turner and Lopez, 1990). The proposal that blood-flow changes in one testis may influence flow in the contralateral testis is also complicated by the observation that the contralateral response apparently occurs only under certain conditions. In this study, the response was statistically significant with only one NPY dose, and, in our previous studies demonstrating vasoconstriction after local unilateral injection of serotonin (Collin et al, 1996a) and endothelin-1 (Collin et al, 1996b), we did not observe any changes in blood flow in the contralateral, uninjected organ. The question of whether and, if so, under what conditions unilateral changes in blood flow induce neuronal reflexes influencing the contralateral testis therefore remains unanswered.

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